deficiency that responds to iron supplementation [14], the anemia of copper deficiency should not be treated by iron supplementation but should be treated by either lowering the intake of dietary iron or by chelation therapy [15–17]. As can be seen in the present study, the less severe anemia of copper deficiency was caused by the consumption of a low-iron diet. In contrast, the most severe anemia in copper-deficient rats was induced by consumption of an additional low-iron diet. In contrast, the most severe anemia in copper-deficient rats was caused by the consumption of a low-iron diet. However, copper deficiency was not severe enough to be induced by the consumption of an additional low-iron diet. This finding may have practical significance to clinicians dealing with cases presenting as anemias of iron deficiency.

References


Electrochemical Enzyme Immunoassay for Serum Prostate-Specific Antigen at Low Concentrations, Sung-Fang Chen, Yan Xu,* and Michael Po-Chee Ip†(Dept. of Chem., Cleveland State Univ., Cleveland, OH 44115 and 1 Dept. of Pathol., MetroHealth Med. Center, Cleveland, OH 44109; *author for correspondence: fax 216-687-9298, e-mail y.xu@popmail.csuohio.edu)

Serum prostate-specific antigen (PSA) has been recognized as a sensitive indicator of recurrent prostate cancer after radical prostatectomy [1–5]. In the past 5 years, numerous PSA assays with improved limits of detection [6–15] have been developed by both clinical researchers and diagnostic assay manufacturers. The rationale behind the development of more sensitive PSA assays (e.g., lower limits of detection) is that the relapse of prostate cancer or the tumor-doubling time after radical prostatectomy can be detected much earlier if patients are monitored with more sensitive assays [16, 17].

Here we report a rapid enzyme immunoassay (EIA) for serum PSA at low concentrations by flow-injection electrochemical detection, which is based on the modification of the Tandem-E®-PSA assay (Hybritech, San Diego, CA). EIA coupled with electrochemical detection offers low limits of detection with high specificity [18–21]. Electrochemical EIA is usually based on the conversion of an electroinactive substrate to an electroactive product by the enzyme label. In this work, the enzyme was alkaline phosphatase (EC 3.1.3.1), which converted p-aminophenyl phosphate to p-aminophenol [22]. The concentration of p-aminophenol was then determined amperometrically in the flow-injection system.

We had illustrated the flow-injection electrochemical detection system used for this work elsewhere [21]. Basically, it was a BAS chromatograph (Bioanalytical Systems, West Lafayette, IN) without the separation column. The thin-layer electrochemical cell had dual glassy carbon working electrodes (in the parallel mode), a Ag/AgCl (3 mol/L NaCl) reference electrode, and a stainless-steel auxiliary electrode. The detection voltage between the working and reference electrode was set at +500 mV. The system had a custom-built injector with 1-μL sample loop. The carrier fluid (0.1 mol/L Tris, 1 mmol/L MgCl₂, 65 mmol/L oxalic acid, and 0.2 g/L NaN₃ at pH 3.2) was pumped at 0.2 mL/min.

In our assay procedure, Tandem-E® PSA assay kit was used. A set of low-concentration PSA calibrators (0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μg/L) was prepared through serial dilutions of the PSA calibrators (2, 10, and 50 μg/L) with the zero diluent. The assay was carried out in duplicate as follows: (a) Pipette 100 μL of the zero diluent, calibrators, controls, and serum specimens, as well as 100 μL of anti-PSA antibody–alkaline phosphatase conjugate, into each appropriately labeled test tube; (b) add one anti-PSA antibody-coated bead to each tube and vortex-mix the tubes; (c) shake the test tube rack gently in the water bath of the shaking incubator at room temperature (~23 °C) for 2 h; (d) aspirate the liquid from the tubes and wash the beads with the wash solution (4 × 1 mL); (e)
place each bead into a fresh test tube containing 200 μL of 4 mmol/L p-aminophenyl phosphate (in 100 mmol/L Tris and 1 mmol/L MgCl₂, pH 9.0) and incubate for 5 min at room temperature; (f) pipette 30 μL of 0.5 mol/L oxalic acid into each tube to stop the enzyme reaction (oxalic acid lowers the pH from 9.0 to 3.2) and vortex-mix; and (g) draw the solution from each tube with a syringe and inject the sample into the flow-injection system, where the enzyme product, p-aminophenol, is detected by an amperometric detector.

With our electrochemical EIA procedure, a six-point calibration curve for serum PSA was constructed with oxidative currents of p-aminophenol vs PSA concentrations (Fig. 1A). The calibration curve had a linear dynamic range from 0.02 to 1.0 μg/L with a correlation coefficient of 1.00 and CVs <5.5% over its range. The limit of detection (LOD) of the method was calculated to be 0.008 μg/L, which was defined as the mean signal (n = 19) of the zero diluent + 2 SD. Compared with the Tandem-E PSA assay, which has a LOD of 0.3 μg/L, our assay lowered the LOD by >37-fold.

For serum sample analysis, two PSA calibrators and two PSA controls (which were prepared by serial dilutions of the high-concentration PSA controls provided in the assay kit with the zero diluent) were used. Because there is no internationally accepted PSA reference standard available, we could not study the accuracy of our assay procedure. However, comparing the results of our assay with the Tandem-E PSA assay performed on the Photon ERA® automated immunoassay analyzer (Hybritech) was informative. We carried out a comparison study with serum samples containing PSA ranging from 0.235 to 4.80 μg/L. Because a lower LOD (0.008 μg/L) was obtained by our assay procedure, a 10-fold dilution of sample with the zero diluent was performed before the analysis. The results of our electrochemical EIA were plotted against the results of the Tandem-E PSA assay by a factor of 0.1 (Fig. 1B). On 16 patients’ samples, a good correlation (r = 0.999) was obtained between these two assays, and no bias was observed (m = 1.00).

In conclusion, the electrochemical EIA of PSA offers a much lower LOD (0.008 μg/L) and requires shorter enzyme reaction time (5 min) than those of the Tandem-E PSA assay (LOD 0.3 μg/L, enzyme reaction time 30 min). It has a potential for use in small-volume analysis (only 1 μL sample was injected for final detection), and its LOD may be further improved by increasing the enzyme reaction time.

This work was supported by a research grant from the American Association for Clinical Chemistry.

References

Fig. 1. (A) Calibration curve of electrochemical EIA with 1-μL sample injection; (B) linear regression analysis of Tandem-E PSA assay vs electrochemical EIA.
Prostate-Specific Antigen in Ascitic Fluid, Ferdinando Mannello, Giovanni Miragoli, Giuseppe Bianchi, and Giancarlo Gazzanelli

Although previously thought to be produced almost exclusively by the epithelial cells of the prostate [11], prostate-specific antigen (PSA) is produced and secreted by several extraprostatic sources [2, 3]. Considering the high degree of homology of PSA with the human glandular kallikrein (hKGG1) [4, 5], and the activation of the kallikrein–kinin system in peritoneal effusions [6, 7], we undertook the study of PSA distribution and expression in ascitic fluids. Between May 1996 and January 1997 we collected ascitic fluids from 44 consecutive women ages 29–61 years (mean 49 ± 7) undergoing ultrasound examination and a diagnostic paracentesis. After collection (~10 mL), ascitic fluids were centrifuged at 20 120 g for 20 min at +4 °C and the supernatants stored at −30 °C until processed. Blood samples were also taken, and after clotting were centrifuged at 360 g for 5 min at +4 °C and stored at −30 ºC until assay. In 24 patients (ages 28–81 years), the ascites was associated with malignancies (ovarian, pancreatic, breast, gastrointestinal, and lung). In the other 20 patients (18–75 years), ascites arose from chronic liver diseases, bacterial peritonitis, congestive heart failure, thrombosis, and other nonmalignant diseases. Albumin, serum–ascites albumin concentration gradients, total protein, lactate dehydrogenase, and cholesterol concentrations were also measured in the fluids (data not shown) [8]. PSA was measured by two methods [9–11]: a solid-phase two-site IRMA (PSA-RIACT™) from CIS Bio International (Gif-sur-Yvette, France) and a microparticle capture enzyme immunoassay (MEIA) (IMX®) from Abbott Labs, Abbott Park, IL. Patients with malignant ascites had not yet received cytotoxic drugs and (or) chemotherapeutic agents before sample collection. Results are expressed as means ± SE. Statistical analyses were performed through the StatView v.4.1 package (Abacus Concepts, Berkeley, CA) on Macintosh Power PC (Apple Computer, Cupertino, CA).

The work was carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983. Among the 44 patients examined, 41% of ascitic fluids contained detectable amounts of PSA, tested with both assay methods (mean ± SE 0.278 ± 0.045 µg/L, range 0.06–0.78 µg/L, n = 18). Matrix effects of ascitic fluid constituents in the PSA assays were excluded by performing dilutions of samples having high PSA content. A good linearity (r = 0.973) between PSA content and dilution was obtained with the IMx method. The PSA-RIACT (y) and IMx-PSA (x) agreed (n = 15, y = 0.019 + 0.863x, means ± SE of y = 0.251 ± 0.068 and of x = 0.268 ± 0.077, r² = 0.958, P <0.0001). In agreement with others [12, 13], we found a plasma PSA content =0.05 µg/L in ~91% (40 of 44) of the women examined. The PSA mean concentration in our series of ascitic fluid samples did not show a significant difference between malignant-related and nonmalignant ascitic fluids (0.262 ± 0.074 µg/L, 0.297 ± 0.047 µg/L, respectively; t = −0.317271, P = 0.761). The total PSA content in ascitic fluids was significantly greater than in plasma (0.278 ± 0.045 µg/L and 0.032 ± 0.011 µg/L, respectively; n = 18, P <0.0001). PSA was not statistically significantly correlated with patient’s age.

A major 33-kDa immunoreactive band (due to the free form of this serine protease) was seen on Western blots with an anti-human PSA monoclonal antibody (Dako, Milan, Italy). The 100-kDa immunoreactive protein due to the α1-antichymotrypsin-bound form was not detected, nor were other bands (Fig. 1).

Several sources could be suggested for PSA expression in ascitic fluids: (a) plasma ultrafiltration and accumulation, at an increased rate in the peritoneal space through a vascular hyperpermeability of the inflamed peritoneal tissue. Previous data have shown the liberation, accumulation, and activation of the kallikrein–kininogen–kinin system [6, 7] and the role of a vascular permeability factor in the pathogenesis of ascitic fluid accumulation [14], (b) Local secretion mainly due to the enhanced protease synthesis by the neoplastic ascitic cells. Several reports have documented the activity of proteolytic enzymes in peritoneal fluid [6, 15, 16] as well as in an experimental